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Research Article

The abundance of capsule (wabG) and fimbria (fimH) coding genes in carbapenem-resistant *Klebsiella pneumoniae* strains isolated from patients admitted to Isfahan hospitals

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ABSTRACT

Infection by carbapenem-resistant strains of *Klebsiella pneumoniae* is a life threating problem in hospitals. Capsule and fimbriae are known as important virulence factors for *Klebsiella pneumoniae*. The aim of this study was to investigate the prevalence of two genes coding capsule (*wabG*) and fimbriae (*fimH*) in carbapenem-resistant *Klebsiella pneumoniae* strains isolated from patients hospitalized in Isfahan, Iran. Disk diffusion was used to test the isolates' susceptibility to carbapenem. The carbapenem-resistant isolates were definitively confirmed using the 16S–23S ITS gene; ultimately, carbapenem-resistant (OXA48), capsule (wabG), and fimbriae (fimH) genes were found in the isolates. The data were analyzed using an independent parametric T test and one-way analysis of variance (ANOVA). Out of 102 clinical isolates, 50 isolates (49%) were carbapenem resistant and 52 isolates (51%) were sensitive to carbapenem. The most carbapenem-resistant isolates were isolated from the ICU (42%) and emergency departments (22%). All carbapenem resistant isolates had the *OXA48* gene. Out of 50 isolates with carbapenem resistance gene, 48 isolates (96%) had *fimH* gene and 47 isolates (94%) had *wabG* gene. Most carbapenem resistant isolates with *fimH* were from men (31 isolates), and from respiratory trachea (31%) and urine (27%). Most carbapenem resistant isolates with *wabG* were from men (29 isolates), respiratory trachea (28%) and urine (30%). The high frequency of carbapenem resistance *in Klebsiella pneumoniae* isolates, as well as the high frequency (more than 90%) of fimH and wabG genes, is relevant and should be included in diagnostic and treatment regimens.

1. Introduction

Klebsiella pneumoniae is an opportunistic bacteria that causes pneumonia, urinary infections, and severe liver abscesses in humans. Recent studies showed that the prevalence of Klebsiella colonization ranges from 18.8 to 87.7% in Asia and 5 to 35% in Western countries. The rate of transmission of *Klebsiella pneumoniae* in hospitals is related to the length of hospitalization, and the intensity of colonization. Except for contamination caused by faulty hygiene practices in medical equipment and blood products, the main reservoirs of *Klebsiella pneumoniae* transmission in the hospital are the digestive system of patients and the hands of hospital staff. (Liu et al.,2019). .This bacteria is one of the most important pathogens involved with multidrug-resistant (MDR) infections, putting

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treatment at risk. The ability of this organism to spread rapidly often causes hospital-acquired infections. Epidemic nosocomial infections caused by MDR strains are very dangerous (Tan et al., 2020; Baker, 2016). Unfortunately, uncontrolled antibiotic usage in recent decades has resulted in the rise of antibiotic-resistant *Klebsiella pneumoniae* strains. Although several antibiotics are used to treat *Klebsiella pneumoniae* infections, carbapenems, such as meropenem and imipenem, are the cornerstone of treatment for severe infections with this bacteria. However, many strains have developed resistance to these medicines, making treatment of infections caused by this organism difficult (Reyes et al., 2019).

One of the important mechanisms for the resistance of Gram-negative bacteria to carbapenems is the production of carbapenemase enzymes. OXA-48 which is a class D carbapenemase, is a major concern for antibiotic resistance due to its difficulty in diagnosis and its association with treatment failure. In addition, OXA-48 is encoded in a plasmid and is therefore associated with the rapid spread of antibiotic resistance among Gram-negative bacterial pathogens (Evan and Amyes, 2014).

Fimbriae, capsule, lipopolysaccharide (LPS), outer membrane proteins (OMPs), and iron transfer agents (Sidrophores) are four important virulence factors in *Klebsiella pneumoniae* (Zhu et al., 2021; Gan et al., 2022).

Genes encoding virulent factors in *Klebsiella pneumoniae* are the main cause of the damage caused by this bacterium. These genes include *mag*, *armp*, *armpA2*, and *allS*, which are associated with hypermucoviscosity and mucoviscosity phenotypes; *wabG*, uge, and wcaG genes, which are involved in lipopolysaccharide biosynthesis; *iutA*, *icuA*, *iroN*, *iroB*, *ybtA*, *irp2*, *kfu*, and *entB*, which are involved in iron absorption; and *Cf29a*, *fimH* and *mrkD* genes, which are related to adhesion (Effah et al., 2020). Fimbriae are encoded by the *fim* gene, which includes all the genes required for fimbrial construction and assembly. The main component of the fimbrial appendage consists of repeating subunits of Fima and an adhesion molecule (Fimh) at the tip of the fimbria that binds to mannose-containing glycosidic receptors on host cells (Murphy et al., 2013). *fim* genes have been detected in strains resistant to carbapenem antibiotics in recent studies (Makhrmash et al., 2022; Jin et al., 2022). The *wabG* gene is also known as one of the most common genes found in invasive and carbapenem-resistant *Klebsiella pneumoniae* strains in recent studies (Hasani et al., 2020; Fursova et al., 2021).

Considering the increasing resistance to carbapenems in virulent strains of *Klebsiella pneumoniae*, the present study aims to investigate this resistance in *Klebsiella pneumoniae* strains isolated from patients admitted to hospitals in Isfahan, Iran, and to trace the LPS coding gene (*wabG*) and the fimbriae coding gene (*fimH*) in these strains.

2. Materials and Methods

2.1. Samples

A number of 102 isolates of *Klebsiella pneumoniae* were collected from 200 clinical samples (lung, blood, urine, and purulent secretions) in different departments of hospitals in Isfahan, Iran from March 2022 to July 2022 and were transferred to the microbiology laboratory for verification. The bacterial samples were cultured on the two basic media of blood agar and McConkey agar. After ensuring the purity of the colonies, the isolates were examined using microbiological and biochemical tests related to the identification of *Klebsiella pneumoniae*. After extracting the DNA of bacterial isolates by boiling, the definitive confirmation was done by the amplification of a specific 130 bp fragment in the 16S-23S internal transcribed spacer (16S-23S ITS) gene of *Klebsiella pneumoniae* with PCR, by using the pair of primers listed in Table 1. The thermal program used included a step of 94 °C for 5 min; 35 repeated steps of 95 °C for 30 s, 58 °C for 90 s, and 72 °C for 90 s; and a final step of 72 °C for 10 min (Turton et al., 2010). *Klebsiella pneumoniae NCTC 9633* was Positive and water was used as negative control. The PCR products were visualized in 1% agarose gel electrophoresis.

2.2. Antibiotic sensitivity test

To isolate bacteria with antibiotic resistance, disc diffusion method was performed using meropenem (MEN, 10 µg), imipenem (IPM, 10 µg), ciprofloxacin (CP, 5 µg), gentamicin (GM, 10 µg), and cotrimoxazole (SXT, 10 µg)

antibiotic discs. The results were interpreted according to the diameter of the growth inhibition zone of each antibiotic disk by referencing to the standard tables of Clinical and Laboratory Standard Institute (CLSI). Isolates resistant to carbapenems were used for the next stages of the research.

2.3. Molecular verification of carbapenem resistant bacteria using OXA-48 gene

For molecular confirmation of carbapenem resistant bacteria, PCR of OXA-48 gene was performed using the specific primers of this gene listed in Table 2. (Shoja et al., 2018). Positive *control Escherichia coli ATCC 25922*, and Negative control were Water.

2.4. Investigating the presence of virulence genes in carbapenem resistant isolates

To check the presence of *fimH* and *wabG* genes, the PCR method was used on the extracted DNA using the primers listed in Table 3. The thermal program used included a step of 94 °C for 5 min; 35 repeated steps of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final step of 72 °C for 5 min (IN Study).

2.5. Data analysis

The data results were statistically analyzed using SPSS version 20 (Chicago SPSS, USA) and Excel 2010 (Microsoft Corporation, USA). The difference between the averages and the determination of the relationships were calculated using the statistical methods of the parametric independent T test and the analysis of variance (one-way ANOVA). The p value < 0.05 was considered statistically significant.

Table 1. The sequences of primers used for definite identification of *Klebsiella pneumoniae* strains among the isolates

Target gene	Sequences of primers (5′→3′)	Amplicon size (bp)	Reference
16S-23S ITS	F: ATTTGAAGAGGTTGCAAACGAT R: TTCACTCTGAAGTTTTCTTGTGTTC	130	Turton et al., 2010

Table 2. Primer sequence for genotypic identification of carbapenem resistant bacteria using OXA-48 gene

Target gene	Sequences of primers (5′→3′)	Amplicon size (bp)	Reference
OXA-48	F: TATATTGCATTAAGCAAGGG R: CACACAAATACGCGCTAACC	847	Shoja et al., 2018

Table 3. Primer sequence for genotypic identification of carbapenem resistant strains

Target gene	Sequences of primers (5′ →3′)	Amplicon size (bp)	Reference
fimH	F: TATGGCGGTGTGCTGTCGAG R: GGGAGGGTGACGGTGACATC	329	IN Study
wabG	F: TCCCGGCTGCGATCTCTACC-3' R: CGGAGCCGACGTAGATCAGG-3'	362	

3. Results

3.1. Distribution of the isolates

The present study was conducted on 102 strains of *Klebsiella pneumoniae* isolated from different clinical samples from Isfahan teaching hospitals. After phenotypic identification by biochemical tests, the isolates were definitively diagnosed by tracing *16S-23S ITS* gene. All isolates showed a 130 bp fragment and were diagnosed as positive. The results are shown in Fig. 1.

3.2. Distribution of carbapenem resistant isolates

Out of 102 isolates, 50 isolates (49%) were carbapenem resistant and 52 isolates (51%) were sensitive to carbapenem. All carbapenem resistant strains contained *OXA48* gene. Out of 50 carbapenem-resistant *Klebsiella pneumoniae* isolates, the most isolates were obtained from ICU with 21 samples (42%) and emergency department with 11 samples (22%). All carbapenem-resistant isolates had *OXA48* gene, which confirmed the presence of carbapenemase class D gene sequence in them. The least isolates were obtained from elective operating room, gynecological surgery, orthopedics, Internal medicine, pediatric surgery, Lung, internal general, heart, skin, endocrinology, and gastroenterology departments with 1 sample (2%) each (Fig. 2). Considering the significance of chi-square test, it was concluded that there was a significant relationship between the distribution of resistant isolates and different departments of the hospital.

3.3. Distribution of fimH and wabG genes in carbapenem resistant isolates

Out of 50 isolates with carbapenem resistance gene, 48 isolates (96%) had *fimH* gene (17 isolates from female patients and 31 isolates from male patients) and 47 isolates (94%) had *wabG* gene (18 isolates from female patients and 29 isolates from male patients).

3.3.1. Frequency distribution based on the sample type

The frequency distribution of carbapenem-resistant isolates with *fimH* and *wabG* genes in different samples are shown in Fig. 3. Out of 48 carbapenem resistant isolates (with *fimH* gene), the most isolates were obtained from respiratory trachea with 15 samples (31%) and urine with 13 samples (27%) and the least isolates were obtained from feces, synovial fluid, wound secretions, and direct pleural fluid smear, each with 1 sample (2%). There was a significant difference in the distribution of *fimH* gene in the strains based on the type of samples. Out of 47 carbapenem resistant isolates (with *wabG* gene), the most isolates were obtained from respiratory trachea with 13 samples (28%) and urine with 14 samples (30%) and the least isolates were obtained from feces, synovial fluid, wound secretions, and direct pleural fluid smear, each with 1 sample (2%). There was a significant difference in the distribution of *wabG* gene strains based on the type of samples.

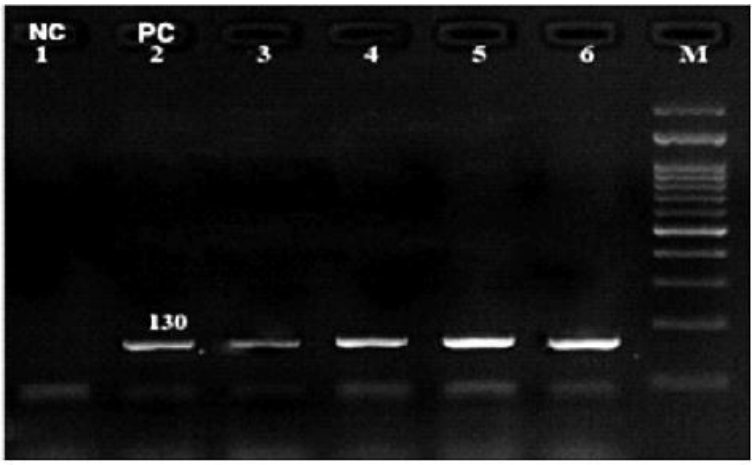

Figure 1. The image obtained from agarose gel electrophoresis of the PCR products related to the detection of the *16S-23S ITS* gene in some *Klebsiella pneumoniae* strains isolated from different clinical samples (M: 100 bp DNA marker, 1: negative control, 2: Positive control, 3-6: some studied clinical isolates with a 130 bp PCR product.

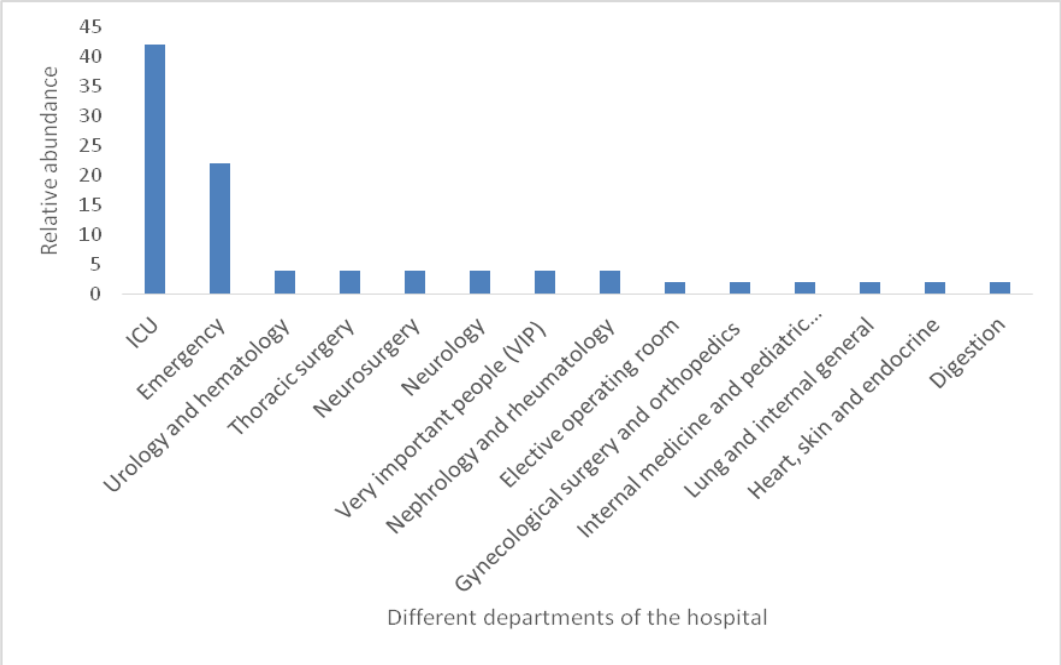

Figure 2. Frequency distribution of resistant isolates based on different departments of the hospital.

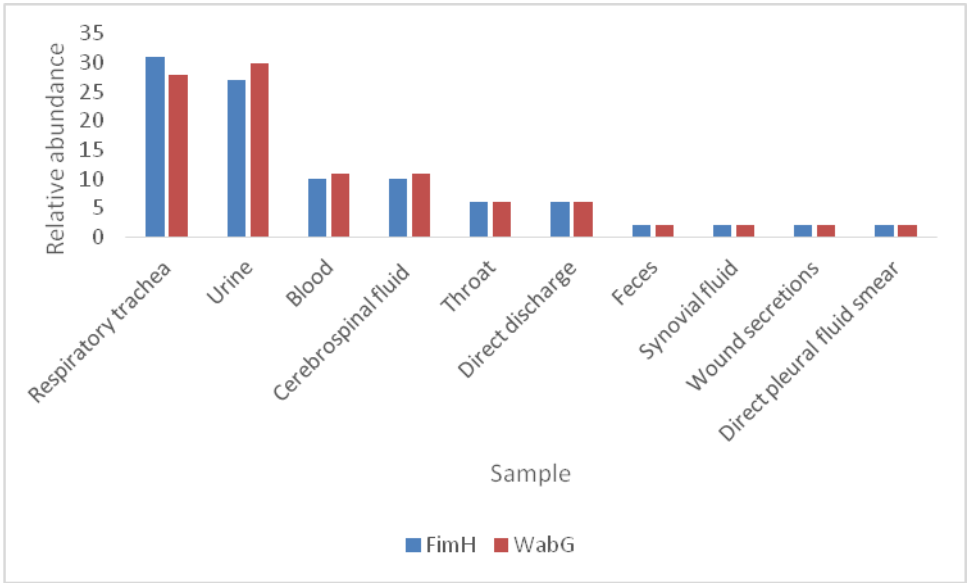

Figure 3. Frequency distribution of carbapenem resistant isolates (with *fimH* and *wabG* gene) according to the samples type.

4. Discussion

The resistance of *Klebsiella pneumoniae* to beta-lactam antibiotics has caused major problems in the treatment of infections caused by this pathogen. Beta-lactamase production plays an important role in this field. Broad-spectrum beta-lactamases in Gram-negative bacteria have been proposed as the most important means of resistance to beta-lactam antibiotics in recent decades. These enzymes are secreted in the periplasmic space in the cell wall of the bacteria, and interface the beta-lactam antibiotic molecules that pass through the outer membrane before reaching their site of action. Although, it seems that all Gram-negative

bacteria contain beta-lactamase enzyme, but the type and amount of these enzymes in bacteria are significantly different. These enzymes can be coded by chromosomal or plasmid genes, and be a part of the bacterial structure or inducible by certain classes of beta-lactam drugs (Hashemi, 2016). In the past decades, carbapenems have been the selected antibiotics for the treatment of patients infected with broad-spectrum β-lactamase-producing strains, although resistance to carbapenems in bacteria is increasing (Reyes et al., 2019; Jin et al., 2022). Out of 102 strains investigated in this research, 50 strains (49%) had carbapenem resistant gene (*OXA48*) and 52 strains (51%) were sensitive to carbapenem. The most carbapenem-resistant strains were significantly isolated from the ICU (42%) and emergency departments (22%), which was similar to other antibiotic-resistant strains in terms of isolation location. Li et al. (2019) found that out of 507 isolates of *Klebsiella pneumoniae*, 244 (48.1%) strains were carbapenem resistant which is coincident with the results of the present study. Infectious diseases in ICU patients may be a challenging condition with a high mortality and an adequate antibiotic coverage should be applied for any possible pathogen. However, the indiscriminate consumption of antibiotics has leaded to an accelerated incidence of antibiotic resistant strains in recent years (Tacconelli et al., 2018).

Out of 50 strains with carbapenem resistance gene, 48 strains (96%) had *fimH* gene and 47 strains (94%) had *wabG* gene. Carbapenem-resistant strains with *fimH* gene were more isolated significantly from respiratory trachea (31%) and urine (27%) samples, which was the same high frequency result of other antibiotic resistant strains in urine samples. Also, among the carbapenem resistance strains with *wabG* gene, the most strains were significantly isolated from the respiratory tract (28%) and urine (30%), which was the same high frequency result of other antibiotic resistant strains in urine samples. Hosseini et al. (2018) collected 65 strains of *Klebsiella pneumoniae* during a descriptive-cross-sectional study. In the molecular analysis, the frequency of *fimH* and *rmpA* genes was reported as 86.1% and 10.8%, respectively, but none of the strains carried the *magA* gene. The frequency of *fimH* gene in their study was lower than that of the present study. Shivaee et al. (2019) identified *Klebsiella pneumoniae* isolates in patients referred to Motahari and Milad hospitals in Tehran (Iran) from October 2015 to June 2016 with biochemical tests. The ability to form biofilm was determined by phenotypic test. Virulence factors were identified by PCR method. The highest resistance to ceftazidime and cefotaxime (67%) and the lowest resistance to imipenem and meropenem (39%) were reported, which is much less than the frequency obtained in the present study. A total of 81% of the strains were able to form biofilm. Also, PCR results showed that all 57 biofilm-forming isolates had *fimA*, *mrkA*, *ecpA*, and *fimD* genes. The *fimH* gene was identified in 92% of the isolates and was not observed in any of the isolates that did not form biofilm. The results of that study showed the importance of investigating the formation of biofilm in resistant strains, and considering the high frequency of *fimH* gene in the strains in the present study, these strains may have a high importance in hospitals. Fursova et al. (2021), also detected the prevalent of virulence genes, *wabG* and *fimH*, in 41% of MDR *Klebsiella pneumoniae* isolates, which were lower than the *allS* (41%), and *uge* (34%). Hasani et al. (2020) also found the common virulence genes including *uge* (93.4%), *ycfM* (91.8%), *wabG* (88.5%), *wcaG* (29.5%), and *rmpA* (21.3%) in clinical isolates. Adhesive elements in *Klebsiella pneumoniae* including fimbriae, capsule, and siderophores are the main virulence factors contributing the pathogenicity of *Klebsiella pneumoniae* strains (Karampatakis et al., 2023). Knowledge of the prevalence of these virulence factors in carbapenem resistant strains of *Klebsiella pneumoniae* is crucial. The studies such as the present study can help to better detection of the prevalence of these strains and the selection of alternative treatment protocols for combatting their infections.

Conclusion

The present study showed a high frequency of carbapenem resistance in multidrug-resistant strains of *Klebsiella pneumoniae* isolated from different clinical samples in Isfahan, Iran. The highest antibiotic resistance and carbapenem resistance were observed in the ICU and emergency departments, and urinary tract and respiratory tract infections showed the highest antibiotic-resistance and carbapenem-resistance.

The frequency of more than 90% of *fimH* and *wabG* genes in the carbapenem resistant isolates, which is higher than many previous reports, is significant and should be considered in diagnostic and treatment protocols.

Conflict of interest

None

Refereces

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